ENVIRONMENTAL POLLUTION

ELSEVIER

Environmental Pollution 120 (2002) 313–317

www.elsevier.com/locate/envpol

Seasonal phosphatase activity in three characteristic soils of the English uplands polluted by long-term atmospheric nitrogen deposition

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Received 24 August 2001; accepted 25 January 2002

"Capsule": *High soil phosphatase activities confirm strong biological phosphorus limitations due to nitrogen deposition.*

Abstract

Phosphomonoesterase activities were determined monthly during a seasonal cycle in three characteristic soil types of the English uplands that have been subject to long-term atmospheric nitrogen deposition. Activities (μmol *para*-nitrophenol g^{-1} soil dry wt. h^{-1}) ranged between 83.9 and 307 in a blanket peat (total carbon 318 mg g^{-1}, pH 3.9), 45.2–86.4 in an acid organic grassland soil (total carbon 354 mg g^{-1}, pH 3.7) and 10.4–21.1 in a calcareous grassland soil (total carbon 140 mg g^{-1}, pH 7.3). These are amongst the highest reported soil phosphomonoesterase activities and confirm the strong biological phosphorus limitation in this environment. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Atmospheric nitrogen deposition; Peat; Phosphatase; Phosphomonoesterase; Phosphorus; Soil; Uplands

1. Introduction

Atmospheric nitrogen (N) deposition can cause or enhance biological phosphorus (P) limitation in natural and semi-natural ecosystems (Aber et al., 1989). This increases the importance of organic P compounds to plants and microorganisms and accelerates the synthesis of enzymes capable of releasing orthophosphate from such compounds (Johnson et al., 1998; Turner et al., 2001). The dominant phosphatase enzyme in most soils is phosphomonoesterase (PMEase), a relatively non-specific phosphohydrolase that acts on a range of low molecular weight P compounds with a monoester bond, including mononucleotides, lower inositol phosphates, sugar phosphates, and polyphosphates (Reid and Wilson, 1971). Therefore, the activity of PMEase would be expected to play an important role in organic P turnover in soils under elevated atmospheric N deposition and reflect the prevailing degree of P limitation. Our aim was to determine PMEase activities during a seasonal cycle in three contrasting soils characteristic of the English uplands polluted by long-term atmospheric N deposition.

2. Methods

The Upper Teesdale National Nature Reserve was chosen as the study area, because it has received substantial levels of atmospheric pollutants, including reactive N compounds, since the industrial revolution, and contains relict late-glacial plant assemblages that are sufficiently rare to be of international importance (Clapham, 1978). Some plant communities are strongly limited by P availability, but it is difficult to assess whether atmospheric pollution has caused or enhanced this effect (Turner et al., 2001). There are three distinct soil types and related plant communities: blanket peat, dominated by *Calluna vulgaris*, *Erica tetralix* and *Sphagnum* spp.; acid organic soils under grassland, dominated by *Festuca ovina* and *Nardus stricta*; calcareous soils under grassland, dominated by *Kobresia*

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0269-7491/02/$ - see front matter
PII: S0269-7491(02)00147-1

simpliciuscula, *Carex ericetorum* and *Thymus praecox* ssp. *arcticus* (Clapham, 1978). The grasslands are grazed by sheep. There have been floristic changes in the area since the 1960s, notably reductions in bryophyte diversity and lichen abundance (Huntley et al., 1998). Changes in microclimate induced by the creation of a nearby reservoir may have influenced some species, but almost certainly do not explain all the changes.

Soil samples were all collected from Widdybank Fell (O/S Grid Ref. NY 820 300, latitude 54°40′ N, longitude 2°15′ W, maximum height above sea level 519 m, mean annual rainfall 1560 mm), a sufficiently small area to eliminate differences in climate. Mean daily temperatures range from an average of 0.1 °C in February to 12.3 °C in July. Current rates of N deposition onto Great Dun Fell, close to Widdybank Fell, are 20–40 kg ha^{-1} $year^{-1}$ depending on altitude (Hicks et al., 2000).

Soils were sampled monthly between November 1999 and October 2000. On each sampling date, five replicate cores to 5 cm depth were taken randomly from a 5×5 m representative area on each soil type. Soils were bulked and roots, stones, litter and macrofauna removed by hand. All soils unavoidably contained some fine roots, which can contribute to the measured PMEase activity. The calcareous grassland soil was also sampled on 11 occasions during the spring period (19 March to 31 May 2000) to investigate short-term variability between the monthly samples. On one occasion, samples were also taken at 5–10 cm depth. Soil physical and chemical properties are presented in Table 1.

Phosphomonoesterase activity was determined using *para*-nitrophenyl phosphate (*p*NPP) as an analogue orthophosphate monoester substrate (Tabatabai, 1994). Moist soil (0.25 g for blanket peat and acid grassland soil, 0.5 g for calcareous grassland soil) was weighed into glass snap-cap vials and incubated for 30 min in a shaking water bath at 37 °C with 4 ml 0.5 M *Tris*-maleate buffer and 1 ml 50 mM *p*NPP (10 mM final substrate concentration). The buffer contained 0.5 M Tris-hydroxymethyl (aminomethane) and 0.5 M maleic acid, and was adjusted with HCl to pH 6.0 (optimum pH for PMEase activity in the soils). The assays were terminated by adding 1 ml 0.5 M $CaCl_2$ and 4 ml 1 M NaOH. The mixtures were centrifuged for 10 min at 3000×g, then 0.5 ml of the supernatant was diluted in a 25-ml volumetric flask and the absorbance of the released *para*-nitrophenol (*p*NP) measured at 410 nm. Each value was corrected for a blank (substrate added immediately after the addition of $CaCl_2$ and NaOH) and for *p*NP adsorption during the assay (Vuorinen, 1993). Results are expressed on a dry soil basis and also on a soil volume basis to allow comparison between the three soils of dissimilar bulk densities (Harrison, 1979).

3. Results

Monthly phosphomonoesterase activities were greatest in the blanket peat and smallest in the calcareous grassland soil (Table 2, Fig. 1). Differences in PMEase activity amongst the soils were smaller when activity was expressed on a soil volume basis, due to the large variations in bulk density (Table 2). Activities were around 50% lower at 5–10 cm depth compared to 0–5 cm (Table 3).

A clear seasonal trend in PMEase activity was apparent in the blanket peat, being greatest in winter (October–February) and smallest in the spring/summer (March–September; Fig. 1). This variation was largely explained by moisture content ($R^2=0.81$, $P<0.001$, Fig. 2). In contrast, PMEase activities in the acid and calcareous grassland soils were greatest in the spring and early summer (Fig. 1). No significant relationships existed between PMEase activity and soil moisture content for these two soils, nor with pH in all three soils. During the 2 months of weekly sampling, PMEase activity in the calcareous grassland soil varied between 9.97 and 17.5 μmol *p*NP g^{-1} h^{-1} (Fig. 3); these variations were unrelated to pH or moisture content.

Table 1
Physical and chemical properties of the three soils from Widdybank Fell, Upper Teesdale, northern England[a]

Soil type	Sample depth (cm)	pH (water)	Bulk density (g cm^{-3} soil)	Total C	Total N	Total P[b]
				mg g^{-1} soil		
Blanket peat	0–5	3.9	0.306	318	16.7	0.617 (96)
	5–10	3.7	0.242	266	11.9	0.575 (96)
Acid grassland soil	0–5	3.7	0.234	354	24.4	1.233 (94)
	5–10	3.4	0.152	320	20.5	1.217 (95)
Calcareous grassland soil	0–5	7.3	0.566	140	8.8	0.679 (91)
	5–10	7.4	0.445	100	7.0	0.612 (90)

[a] Soils were sampled during September 2000.
[b] Values in parentheses are % organic P.

Table 2
Summary of phosphomonoesterase activities during an annual cycle in the 0–5 cm layer of blanket peat, acid organic grassland soil and calcareous grassland soil from Widdybank Fell, Upper Teesdale, northern England[a]

Soil type		Dry soil basis (μmol *p*NP g^{-1} h^{-1})	Soil volume basis (μmol *p*NP cm^{-3} h^{-1})
Blanket peat	Mean	169.3±58.2	51.8±17.8
	Range	83.9–306.9	25.7–93.9
Acid grassland soil	Mean	61.9±13.0	15.1±3.05
	Range	45.2–86.4	10.6–20.2
Calcareous grassland soil	Mean	15.3±3.37	8.66±1.91
	Range	10.4–21.1	6.48–12.0

[a] Data are expressed on the basis of dry soil (μmol *para*-nitrophenol g^{-1} h^{-1}) and soil volume (μmol *para*-nitrophenol cm^{-3} h^{-1}) and are means±standard deviation of 12 monthly samples.

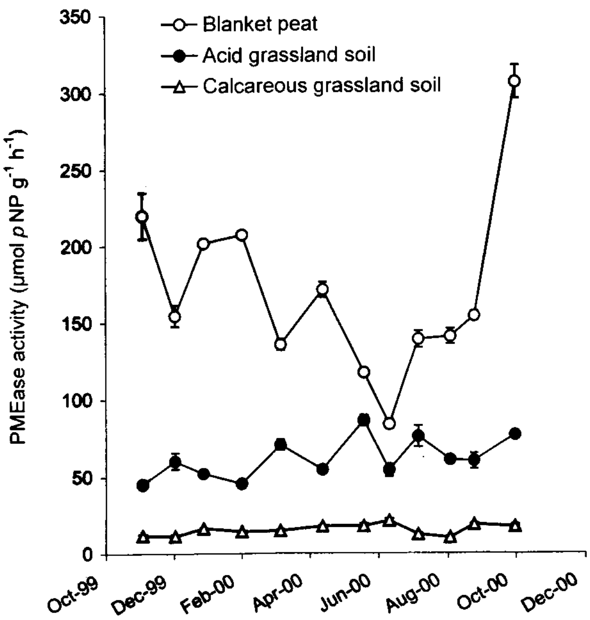

Fig. 1. Seasonal phosphomonoesterase activities (μmol *para*-nitrophenol g^{-1} h^{-1}) in blanket peat, acid grassland soil and calcareous grassland soil from Widdybank Fell, Upper Teesdale, northern England. Values are means±standard error of four replicate assays.

4. Discussion

The PMEase activities in the blanket peat and acid grassland soil reported here are amongst the highest in the literature. Although there is apparently no means of assessing phosphatase data for soils prior to the industrial revolution, it seems likely that atmospheric N deposition on Widdybank Fell has enhanced biological P limitation. Activities were in the range of those reported for three similar soils under atmospheric N deposition in the southern Pennines, England, where PMEase activities determined at 37 °C and soil pH were 38.8 (heathland soil), 123 (acid grassland soil) and 47.9 (calcareous grassland soil) μmol *p*NP g^{-1} h^{-1} (Johnson et al., 1998). When these soils were amended with additional N as ammonium nitrate, activities increased to 119, 166 and 55.1 μmol *p*NP g^{-1} h^{-1} for the same three soils, respectively. For comparison, PMEase activities determined under optimum assay conditions in high organic matter soils of New Zealand (90–461 mg g^{-1} organic C), relatively uninfluenced by atmospheric N deposition, ranged between 12.2 and 43.2 μmol *p*NP g^{-1} h^{-1} (Sarathchandra and Perrott, 1981; Sarathchandra et al., 1984), whilst lower activities equivalent to between 5.1 and 10.6 μmol *p*NP g^{-1} h^{-1} were detected over an annual cycle in a Welsh bog using fluorogenic substrate at field temperature and pH (Kang and Freeman, 1999). The conditions used in the assay of soil phosphatase activity vary depending on the objectives of the assay, but it should be noted that activity determined at soil temperature and pH will generally be lower than that determined under standardised (optimum) conditions.

The high PMEase activities in the soils studied here are probably due to a combination of strong biological P limitation under long-term atmospheric N deposition and PMEase immobilisation to soil organic matter. Soil PMEase activity is derived from a range of sources. Plant tissue can contain high acid PMEase activities under enhanced P limitation (Turner et al., 2001), whilst bacteria seem to be the main microbial source in upland acid soils (Bardgett and Leemans, 1996). Phosphatases released to the soil from plants and microbes can become stabilised by adsorption to humic compounds, which protects them from degradation whilst retaining a small degree of activity (Rao et al., 1996). This stabilisation is reflected in the greater PMEase activities of the two acidic organic soils compared to the calcareous grassland soil and can produce high PMEase activities where P limitation is not enhanced by atmospheric N deposition. For example, average seasonal PMEase activities between 242 and 318 μmol *p*NP g^{-1} h^{-1} were reported from four high altitude (860–1085 m) acid peat soils in North Wales (Bardgett and Leemans, 1996), although these high activities partly reflect the inclusion of litter layers in the assays, which tend to display

Table 3
Phosphomonoesterase activity at two depths in the three soil types sampled during September 2000 from Widdybank Fell, Upper Teesdale, northern England[a]

Soil type	Dry soil basis (μmol *p*NP g^{-1} h^{-1})		Soil volume basis (μmol *p*NP cm^{-3} h^{-1})	
	0–5 cm	5–10 cm	0–5 cm	5–10 cm
Blanket peat	154±2.89	52.7±1.86	47.2±0.89	12.8±0.45
Acid grassland soil	60.2±4.97	30.6±2.32	14.1±1.16	4.65±0.35
Calcareous grassland soil	18.8±0.50	15.0±0.60	10.7±0.28	6.68±0.27

[a] Activities are expressed on the basis of dry soil (μmol *para*-nitrophenol g^{-1} h^{-1}) and soil volume (μmol *para*-nitrophenol cm^{-3} h^{-1}) and are means±standard error of four replicate assays.

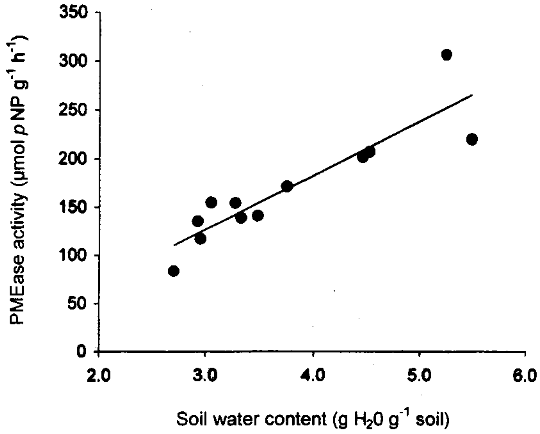

Fig. 2. Relationship between phosphomonoesterase activity (μmol *para*-nitrophenol g^{-1} h^{-1}) and soil moisture content (g H_2O g^{-1} soil) in a blanket peat sampled between November 1999 and October 2000 from Widdybank Fell, Upper Teesdale, northern England. The regression model is described by the equation [PMEase activity] = 56±8.5[soil water content]−41±33; $R^2=0.81$, $F=42.8$, $P<0.001$, $n=12$.

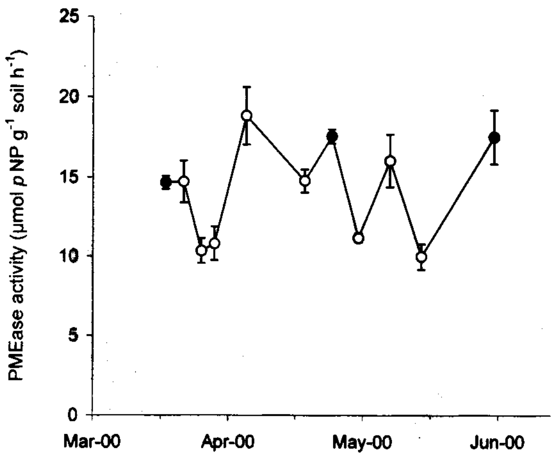

Fig. 3. Phosphomonoesterase activity (μmol *para*-nitrophenol g^{-1} h^{-1}) in a calcareous grassland soil from Widdybank Fell, Upper Teesdale, northern England, during the spring period between 19 March and 31 May 2000, demonstrating the strong temporal variation between monthly sampling dates (shaded circles). Values are means±standard error of four replicate assays.

markedly greater activities than the soil layers. Soil bulk density can also markedly affect the measured PMEase activity and may have contributed to the high measured activities of the two organic soils in the current study.

The maximum PMEase activities in the acid organic grassland soil and the calcareous grassland soil occurred in the spring/early summer, which is in agreement with other studies of UK upland soils (e.g. Bardgett and Leemans, 1996; Kang and Freeman, 1999). This would be reinforced by the variation in phosphatase activity with assay temperature (Harrison and Pearce, 1979) and is consistent with the apparent degradation of some organic P compounds during the summer months in these soils (Turner et al., 2002). However, there was wide fluctuation between sampling dates and it is unclear whether this reflects 'real' temporal variability in PMEase activity with changes in moisture, pH and temperature, or simply the inherent spatial variability in PMEase activity of upland soils (Bardgett and Leemans, 1996; Harrison, 1979).

Despite the high PMEase activities, various organic and inorganic P compounds are present in these soils, including orthophosphate monoesters and diesters, phosphonates and inorganic pyrophosphate (Turner et al., 2002). Some of these compounds should be amenable to phosphatase hydrolysis, suggesting that stabilisation in the soil protects them from enzymatic attack. Organisms able to solubilise such compounds (e.g. through association with mycorrhizal fungi or by excreting organic anions) would have a competitive advantage under enhanced P limitation, which may partly explain observed shifts in the species composition of Upper Teesdale plant communities (Huntley et al., 1998).

Acknowledgements

This work was funded by the Natural Environment Research Council. English Nature kindly granted access to Widdybank Fell. The authors thank Andrew Bristow (Institute of Grassland and Environmental Research, UK) for total carbon and nitrogen analysis.

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